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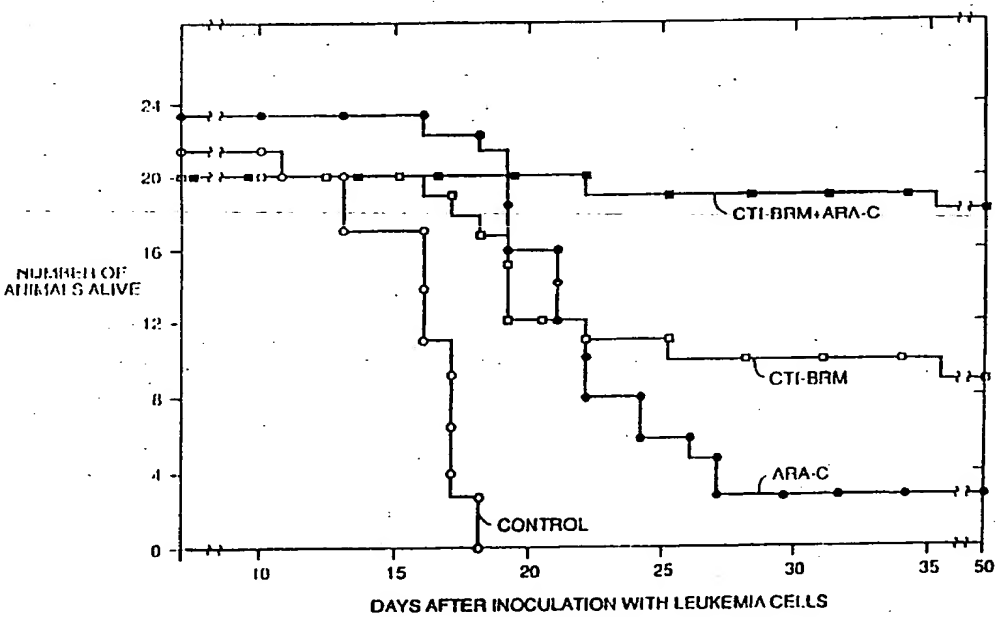
(54) Title: METHOD FOR TREATING CANCER USING COMBINATION CHEMOTHERAPY AND BRM



(57) Abstract

The invention comprises a method for treating tumors in a mammalian patient by administering a combination of a chemotherapeutic agent and a biological response modifier in therapeutically effective amounts to stabilize or substantially reduce the tumor cell load in the mammalian patient. According to the method, a therapeutically effective amount of a chemotherapeutic agent is administered to a mammalian patient, followed by the step of administering the biological response modifier in an amount sufficient to induce substantial terminal differentiation of the residual tumor cells. The biological response modifier comprises natural membrane vesicles and ribosomes in a suspending buffer. The membrane vesicles and ribosomes are derived from a bacterial host. The invention also comprises use of a chemotherapeutic agent and a biological response modifier in the manufacture of a medicament pack for use in the treatment of mammalian tumors.

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METHOD FOR TREATING CANCER USING COMBINATION CHEMOTHERAPY AND BRM

This invention relates to the treatment of tumors in mammals. More particularly, the invention relates to a method for treating a tumor in a mammalian patient by administering a combination of a chemotherapeutic agent and a biological response modifier. In addition the invention relates to use of a chemotherapeutic agent and a biological response modifier in the manufacture of a medicament pack for use in the treatment of mammalian tumors.

BACKGROUND OF THE INVENTION

Various types of chemotherapeutic agents have been demonstrated to be effective in the treatment of tumors in mammalian patients. In many cases, however, the result is merely a reduction in the tumor cell load. Upon cessation of chemotherapy, the tumor often resumes its growth. Since many types of chemotherapeutic agents can be tolerated by a patient for only a limited period of time, this presents a significant problem in treating many types of tumors.

Certain types of tumor cells, particularly various types of leukemias, respond to cytokines by differentiation to different types of mature cells. The ability of cytokines to induce terminal differentiation of leukemia cells suggest the possibility that terminal differentiation of tumor cells may be achieved by administration of cytokines. However, cytokines often demonstrate substantial toxic affects on mammalian patients. Thus far, such problems have limited the effectiveness of cytokines in treating tumors *in vivo*.

It has been discovered that the administration of a biological response modifier derived from the bacterium *Serratia marcescens* is capable of stimulating endogenous differentiation activity production in a mammalian host. Nevertheless, in many instances, the tumor burden may exceed the ability of endogenous differentiation activity to significantly deter further tumor growth.

It is an object of the present invention to provide an improved method for treating tumors in mammalian patients.

Another object of the invention is to provide an improved method for treating tumors in mammalian patients which is more effective than chemotherapeutic agents or biological response modifiers alone.

Still another object of the invention is to provide a method for enhancing the effectiveness of chemotherapy in treating mammalian tumors.

Still another object is to provide medicament packs for use in the treatment of mammalian tumors.

Other objects of the invention will become apparent to those skilled in the art from the following description, taken in connection with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a table illustrating DF and CSF production by stimulated monocytes.

FIGURE 2 is a table illustrating the differentiation inducing activity *in vitro* using one form of the method of the invention.

FIGURE 3 illustrates an experiment in connection with day old Fischer rats demonstrating the effectiveness of the method of the invention in connection with chloroleukemia.

SUMMARY OF THE INVENTION

Very generally, the method of the invention comprises administering a chemotherapeutic agent in a therapeutically effective amount to stabilize or substantially reduce the tumor cell load in the mammalian patient. A biological response modifier is administered in an amount sufficient to induce substantial terminal differentiation of the residual tumor cells. A biological response modifier comprises natural membrane vesicles and ribosomes in a suspending buffer. The membrane vesicles and ribosomes are derived from a bacterial host. The invention also comprises use of a chemotherapeutic agent and a biological response modifier in the manufacture of a medicament pack for use in the treatment of mammalian tumors.

DEFINITIONS

For the purpose of preciseness, the following terms used in this specification and the appended claims are defined:

"Non-toxic" means within a level of toxicity which is tolerable by the mammalian host receiving biological response modifier therapy.

"Non-immunogenic" means evoking a sufficiently low immunogenic response, or no response at all, such that undesired, chronic inflammatory and hypersensitivity responses are not elicited, significantly, in the mammalian host.

"Mean diameter" means the mean diameter of MSD Particle Size Distribution Analysis as measured on a BI-90 (Brookhaven Instrument Corp.) particle sizer. The measurement involves an intensity weighting of the size averaging process and is explained more fully in the Operator's Manual for the instrument, Chapter 6, incorporated herein by reference.

"Substantially non-pathogenic in humans" means not or rarely associated with disease in humans of normal health. Since most microorganisms are capable of causing opportunistic infections under the right circumstances, such as in persons whose immune system has been compromised, this definition excludes only those organism which typically cause non-opportunistic infections.

"Tolerable level of endotoxin, cell walls, and cell membrane fragments" means that any such fractions, if present, have a low enough level of biologic activity to maintain a non-toxic characteristic as defined herein.

"Immune suppressing response" means an immune response which so attenuates the effect of the desired immune response as to be unacceptable for medical purposes.

"Natural membrane vesicles" means membrane vesicles prepared from membranes which are derived from living or dead cells.

"Tumor" means a neoplasm or mass of new tissue which persists and grows independently of its surrounding structures, and which has no physiologic use. *See* Dorland's Illustrated Medical Dictionary, 24th Edition.

Chemotherapy means treatment of tumors by chemical agents, and

a chemotherapeutic agent means a chemical agent used in the treatment of tumors. *See* Dorland's Medical Dictionary, 24th Edition. An example of a chemotherapeutic agent is cytosine arabinoside (ARA-C).

DETAILED DESCRIPTION OF THE INVENTION

The non-specific biological response modifier employed in the method of the invention and its method of manufacture are described in detail in U.S. Patent 4,971,801, issued november 20, 1990 (from U.S. Serial No. 057,344). A corresponding application was published under the Patent Cooperation Treaty as PCT Application PCT/US87/01397. A full and complete description of the biological response modifier and its method for manufacture is contained in the U.S. Patent and the published PCT application sufficient to enable a person having ordinary skill in the art to reproduce the subject material.

For the purposes of this application including the appended claims, the expression "CTI-BRM" shall mean the biological response modifier described in the foregoing application.

CTI-BRM is, at the time of filing this application, undergoing Phase II Clinical Trials for cancer pursuant to regulations of the Food and Drug Administration of the United States of America. Information relating to therapeutically effective amounts of CTI-BRM has been generated in those trials and some of this information is contained in PCT Application No. PCT/US87/01397, as well as in other published articles.

For the purposes of this application, a therapeutically effective amount of CTI-BRM is considered to be substantially equivalent to the amount found to be therapeutically effective in connection with cancer, as set out in the above-mentioned PCT patent application and other publications. A therapeutically effective amount would substantially reduce or would stabilize the tumor cell load in the patient. Such amounts may vary from patient to patient depending on factors such as patient condition, tumor type, etc. Additional variants of therapeutically effective amounts may be readily determinable by the treating physician through observation, and from the information provided herein.

Such are intended to be encompassed within the scope of term "therapeutically effective amount" as used herein and in the appended claims.

Specifically, CTI-BRM comprises natural membrane vesicles and ribosomes in a suspending buffer. The vesicles are comprised of cellular membrane material and are endogenous to a selected organism. The ribosomes are also endogenous to the selected organism. The biological response modifier is substantially free of intact cells, cell walls, and cell membrane fragments. The selected organism is one which does not evoke an immune suppressing response, is non-pathogenic in humans, and is one from which membrane vesicles are capable of being formed from cell membrane material and which vesicles are readily endocytosed by the monocyte macrophage cell line. The vesicle population of the CTI-BRM exhibits a mean diameter of at least about 180 nm on particle size analysis.

Further description of the CTI-BRM is provided in the aforementioned published PCT application. Such description, and the method of preparation, are set forth with particularity in that application and are incorporated herein by reference. The ability of CTI-BRM to alter the levels of various white blood count and neutrophil levels in cancer patients is described in the aforementioned PCT published application.

Dosage regimens described in the aforementioned published PCT patent application included dosage levels ranging from .25 to 10 milligrams administered from 3 to 56 times spaced at 7 day intervals and administered subcutaneously. Toxicity trials indicated no significant toxicity problems with those dosage regimens and further indicated that the product was well tolerated by the human patients. Adjuvant arthritis, granulomas, ulcerations, and similar effects of toxic components are minimized or eliminated by the use of the CTI-BRM.

A preferred source of the material for the CTI-BRM is the organism *Serratia marcescens*. However, other organisms are suitable as source for the membrane vesicles and ribosomes utilized in the CTI-BRM. Such microorganisms should be not a member of the microflora of the patient.

Moreover, the microorganism's common bacterial antigen must not react or at least must be poorly cross-reactive with organisms making up the normal microflora of the patient. Examples of suitable microorganism sources other than *Serratia marcescens* are *Erwinia chrysanthemi* (pectobacterium) and *Enterobacter aerogenes*.

In manufacturing the CTI-BRM, bacterial cells of a strain of microorganism which is not present in the microflora of the patient to be treated and which has a common bacterial antigen which does not cross react or is poorly cross reactive with organisms making up the normal microflora of the patient to be treated, are cultivated. The cultivated cells are harvested and cell membrane is disassociated with an appropriate detergent. The cellular concentrate is subjected to disruption mechanically at a pressure in excess of 10,000 psi to produce membrane vesicles with a mean diameter not less than 180 nm. The membrane vesicles and free ribosomes are separated from the remaining cellular material in the cellular lysate. The membrane vesicles and free ribosomes are then re-suspended in an appropriate buffer.

CTI-BRM is a powerful immunomodulator: it is rapidly phagocytosed by monocytes/macrophages which then show increased phagocytic, bactericidal and tumoricidal activity. Patients injected subcutaneously with CTI-BRM show significant rises in granulocyte counts 24 hours later. Co-culture of CTI-BRM with human peripheral blood mononuclear cells results in elevation of NK activity, increased T-cell mediated cytotoxicity, and augmented lymphocyte and monocyte antibody mediated cytotoxicity (ADCC). The enhancement of these cellular effector functions is most likely a result of a cascade of cytokine release which occurs after CTI-BRM stimulation. Supernatants from CTI-BRM stimulated human peripheral blood mononuclear cells contain IL-1, IL-2, interferons alpha and gamma, TNF and GM-CSF. Both human and rat CTI-BRM stimulated monocytes produce substantial quantities of a myeloid differentiation factor, as measured by the ability to induce differentiation of the rat C51 chloroleukemia *in vitro* and *in vivo*. Jimenez, J.J.,

McCall, C.A., Cirocco, R.E., and Yunis, A.A. 1989. *Journal of Biological Response Modifiers*, Vol. 9, pages 300-304 (1990).

The following examples are set forth to define a specific application of the method of the invention. However, the example set out is not intended to limit the scope of the appended claims.

EXAMPLE I

Dulbecco's modified minimum essential medium (DMEM) was purchased from Flow Laboratories, McLean, VA; horse serum nd fetal calf serum (FCS) were purchased from the University of Miami Comprehensive Cancer Center, Miami, FL. Bacto agar was purchased from Falcon Plastics, Oxnard, CA. Tissue culture dishes were from Corning Glass Works, Corning, NY. Fischer rats were obtained from Charles River Laboratories, Wilmington, MA. Cytosine arabinoside (ARA-C) was purchased from Jackson Memorial Hospital pharmacy, Miami, FL.

Mia C51 is a well characterized cell line established from rat myelogenous leukemia by Yunis, *et al.* *See* Yunis, A.A., Arimura, G.K., Haines, H.G., Ratzan, R.J. and Gross, M.A., Characteristics of rat chloroma in culture. *Cancer Res.* 35:337-345, 1975. It is maintained in DMEM/10% FCS in a humidified incubator at 37°C and 5% CO_2.

Rat peritoneal monocyte conditioned media was prepared as described in Jimenez, J.J. and Yunis, A.A. Treatment with monocyte-derived partially purified GM-CSF but not G-CSF aborts the development of transplanted chloroleukemia in rats. Blood 72:1077-1080, 1988. Fischer rats were sacrificed and the peritoneal cavity was lavaged with serum free (SF) DMEM. The cell suspension at a concentration of 1 x 10^6 monocytes/ml was placed in a tissue culture dish that was placed in a humidified incubator at 37°C and 5% CO_2 for 24 hours. At the end of incubation, the suspension was decanted and to each plate containing the adherent cells SF DMEM was added to which either CTI-BRM (2.5 μg/ml) and/or ARA-C or buffer was also added incubation was continued for 48 hours. The supernatant was then collected, filtered with 0.22

μm filter and dialyzed overnight against double-30 distilled water containing 0.02% Tween 20.

Quantitation of differentiation activity (DA) was obtained using a dispersed colony assay. The C51 cells were seeded in 35 mm petri dishes (cells/plate) containing 1 ml of 0.3% agar in DMEM/10% FCS. The plates were incubated for 3 days and assay samples diluted to 0.2 ml (20% vol/vol) were then added to the incubation mixture. The plates were then incubated at 37°C in a humidified incubator at 5% CO_2 for 7 days. The total number of colonies and the number of dispersed colonies were scored separately; undifferentiated C51 cells formed compact colonies, while differentiation to macrophages was manifested by the appearance of disperse colonies. One unit equals one disperse colony per hundred colonies.

Quantitation of colony stimulating factor (CSF) was achieved using rat bone marrow assay. Briefly, after bone marrow was obtained from the long bones of 6 to 8 week-old rats, cells were diluted with SF DMEM (cell suspension 5×10^6/ml) layered onto a Ficoll-Hypaque density gradient (Histopaque-1077, Sigma Chemical Co., St. Louis) and centrifuged for 30 minutes at 400 g. and the interface cells were then washed twice in SF DMEM; a cell suspension of 5×10^5 cells/ml was prepared in DMEM/10% FCS and incubated in tissue culture dishes for three hours. The nonadherent cells were washed in SD DMEM and used for the bone marrow assay as previously described. One CSF unit equal one colony.

The effect of CTI-BRM and/or ARA-C on the production of DA or CSF by rat peritoneal monocytes was determined.

Supernatants from any peritoneal monocyte cultures prepared in the presence of CTI-BRM (2.5 μg/ml) without or with ARA-C (0.06 μg/ml) demonstrated that ARA-C did not inhibit DF production as measured by the C51 disperse colony assay or CSF production as measured by the rat CFU-GM assay (FIGURE 1). Similarly, monocyte viability was not affected by ARA-C.

Effect of ARA-C and CTI-BRM stimulated monocyte conditioned media (IMCM) on C51 colony formation was also determined.

ARA-C had a dose dependent inhibitory effect on C51 colony formation with maximal inhibition (80% ± 1.5) at 0.06 μg/ml. This inhibition was not associated with differentiation. However, when ARA-C (o.06 μg/ml) was added with IMCM there was a 15% increase of C51 colony differentiation, as compared to IMCM alone (FIGURE 2).

Effect of Treatment with CTI-BRM and/or ARA-C was then determined.

Eighty four 8-day old rats were each injected with 10^5 C51 cells intraperitoneally (IP) and randomized into 4 groups. Group I received 0.1 ml buffer IP daily and served as control. Group II received ARA-C, 20 15mg/kg/day IP. Group III received CTI-BRM 25 μg/ml IP. Group IV received ARA-C, 20 mg/kg/day and CTI-BRM 25 μg/day, both IP. All rats were treated 6 hours after MIA C51 injection for a total of 7 consecutive days. In Group I all rats were dead of chloroleukemia by day 18. In Group II, 2 of 23 (9%) and in Group III 9 of 10 (45%) remained disease-free. In contrast in Group IV 18 of 20 (90%) remained disease-free (FIGURE 3). In Groups II, III and IV, 2(9%), 9(45%), and 18(90%) rats remained disease-free.

In order for CTI-BRM to increase differentiation activity production, it is most likely that at least a portion of the monocyte/macrophage pool in the patient must be functional. Thus, it is significant that the chemotherapeutic agent utilized have little or no effect on the viability of the patient's monocytes. Moreover, the chemotherapeutic agent utilized should not seriously inhibit differentiation activity production in the patient. The foregoing rat study demonstrates significant synergism between the chemotherapeutic agent and the CTI-BRM.

EXAMPLE II

Further evidence of synergism between CTI-BRM and chemotherapeutic agents has been revealed as a result of *in vitro* studies in connection with other chemotherapeutic agents.

195 fresh specimen (SPEC) of human neoplasms (FHN) were cultured for 6 days in the presence and absence of CTI-BRM (30-100 fold concentration range) and then tested for tumor cell specific cytotoxity (TCSC) according to a modification of the DiSC Assay (*Cancer Treat Reporter*, Vol. 70, page 1283). This method constitutes a sensitive and specific method for the assessment of *in vitro* BRM activity in cultures of FHN. The clinical premise is that patients (PTS) with tumors containing endogenous effector cells which are capable of responding to BRM challenge by producing TCSC are more likely to respond to BRM therapy than are PTS whose tumors do not contain such functionally-active effector cells. Differential cell counts performed on 125 solid tumor (ST) SPEC prior to culture showed a medium effector (macrophages + lymphocytes): tumor cell (E:T) ratio 0.5 30% of SPEC lacked discernible lymphocytes, 10% lacked macrophages 35% had an E:T ratio > 1.0 4% had an E:T ratio > 10.0. There was no difference between the E:T ratios of ST biopsies versus effusions. There was no relationship between the activity of any BRM and the E:T ratio (corr. coeff, range 0.02 - 0.18), except for no activity in established cell lines or in fresh tumor SPEC totally devoid of effector cells.

At (1) index (IND) concentrations and at (2) AVG of all concentration tested, the following patterns of disease-specific activity ("sensitive" <50% TC survival) were observed (SPEC "sensitive"/SPEC tested): CTI-BRM (IND = 12 μg/ml); Breast (BR) 2/24-IND, 3/24-AVG; Colon (CO) 2/21-IND, 1/23-AVG, 1/23-AVG; Non-small cell lung cancer (LC) 1/22-IND, 1/19 AVG; Melanoma (MEL) 0/12-IND, 0/13-AVG; Ovary (OVA), 3/26-IND, 4/26-AVG; CLL + NHL + Myeloma (B cell) 4/23-IND, 7/23-avg. IL2 (IND ~166 U/ml); BR 2/22-IND, 3/24 AVG; CO 0/18-IND, 0/20 AVG; LC 1/22-IND, 1/23-AVG; MEL 4/20-IND, 5/21-AVG, OV 4/21-IND, 2/23-AVG; B Cell 0/17-IND, 0/20-AVG. TNGF (IND = 1000 U/ml); BR 0/10-IND, 1/12-AVG; CO 0/12-IND,

0/12-AVG; LC 0/12-IND, 0/12-AVG; MEL 0/9-IND, 0/9-AVG; OV 0/14-IND, 0/18-AVG; B cell 1/15-IND, 1/16-AVG. α-Interferon (α-I-IND = 10,000 U/ml) BR 1/7-IND, 0/7-AVG; CO 3/19-IND, 0/16-AVG; LC 0/10-AVG; MEL 2/12-IND, 0/10-AVG; OV 2/23-IND, 1/18-AVG; B Cell 3/15-IND, 1/15-AVG. Patterns of collateral sensitivity were examined (based on AVG result of all conc. tested): ImV/TNF: = 0.68, P <0.001; ImV/IL2:r = 0.20 n.s.; ImV/α-I = 0.24, p = 0.06; IL/TNF:r =0/05, n.s.; IL2/α-I:r = 0/25,p = 0.05, α-I/TNF = 0.09, n.s.

Detailed analysis of results as a function of previous chemotherapy treatment status showed no significant associations for IL-2, TNF, or α-Inf. In marked contrast, SPEC from previously treated patient had significantly greater overall sensitivity to CTI-BRM, known to activate macrophages and provoke cytokine release. Subset analysis showed that this effect of greater activity against treated tumors held only for chemotherapy responsive tumor histologies (breast, ovary, B cell) and not for unresponsive histologies (colon, lung, pancreas, etc.). Response to chemotherapy may produce an immune-priming effect by increasing macrophage processing of tumor antigens.

It may be seen, therefore, that the invention provides an improved method for treating a tumor in a mammalian patient. A synergistic effect between chemotherapeutic agents and CTI-BRM is noted. It is clear that the method of the invention results in a marked increase in differentiation activity, although the specific nature of such activity is at present not fully understood. Such activity could represent the summation and/or interaction of a number of cytokines. Nevertheless, it is clear that the combination of chemotherapeutic agent and CTI-BRM offers a substantial benefit over each agent alone in the treatment of tumors.

Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treating tumors in mammals, comprising, administering a therapeutically effective amount of a chemotherapeutic agent, followed by the step of administering a therapeutically effective amount of a biological response modifier comprising two major particle populations, one such population being of lesser size particles comprised of ribosomes and the other such population being comprised of natural membrane vesicles in a suspending buffer, said membrane vesicles and ribosomes being endogenous to a selected microorganism which is substantially non-pathogenic in humans, said biological response modifier being substantially free of intact cells, and having tolerable levels of endotoxin, cell walls, and cell membrane fragments.

2. The method of Claim 1 wherein said biological response modifier is derived from the microorganism *Serratia marcescens*.

3. The method of Claim 1 wherein said biological response modifier is administered subcutaneously.

4. The method of Claim 3 wherein said biological response modifier is administered at intervals from 2 to 7 days in an amount between about 0.25 mg and 10 mg.

5. The method of Claim 1 wherein said biological response modifier is administered intraperitonealy.

6. The method of Claim 1 wherein said chemotherapeutic agent comprises ARA-C.

7. The method of Claim 1 wherein in the tumor being treated has a chemotherapy responsive tumor histology.

8. Use of a chemotherapeutic agent and a biological response modifier in the manufacture of a medicament pack for use in the treatment of mammalian tumors.

DF AND CSF PRODUCTION BY MONOCYTES STIMULATED WITH CTI-BRM THE PRESENCE OR ABSENCE OF ARA-C

DRUG (CONCENTRATION IN µg/ml)	VIABILITY (%)	DF (µ/ml)	CSF (µ/ml)
NONE	95	300 ± 20	200 ± 8
CTI-BRM(2.5)	92	36,300 ± 500	2,400 ± 350
ARA-C(0.06)	95	500 ± 32	200 ± 12
CTI-BRM(2.5) + ARA-C(0.06)	88	34,300 ± 2,500	2,600 ± 500

CTI-BRM(2.5 µg/ml) AND ARA-C(0.06 µg/ml) WERE ADDED DIRECTLY TO THE ADHERENT RAT PERITONEAL MONOCYTE POPULATION($1x10^6$ /ml). AT 48 HRS THE SUPERNATANTS WERE COLLECTED, DIALYZED OVERNIGHT AND STERILE-FILTERED. DF UNITS AND CSF UNITS WERE DETERMINED BY ADDING THE SUPERNATANTS DIRECTLY TO THE MIA C51 DISPERSE COLONY ASSAY OR THE RAT CFU-GM ASSAY, RESPECTIVELY.

FIG._1

DIFFERENTIATION-INDUCING ACTIVITY OF IMCM ON MIA C51 LEUKEMIA CELLS IN THE PRESENCE OR ABSENCE OF ARA-C.

DRUG (CONCENTRATION IN µg/ml)	GROWTH INHIBITION (PERCENT)	PERCENT DISPERSED COLONIES
NONE	0	0
ARA-C(0.02)	0	0
ARA-C(0.04)	60 ± 2	0
ARA-C(0.06)	80 ± 1.5	0
IMCM	46 ± 3	45 ± 1
IMCM+ARA-C(0.02)	47 ± 1.5	48 ± 1
IMCM+ARA-C(0.04)	57 ± 5	48 ± 2.5
IMCM+ARA-C(0.06)	80 ± 3	60 ± 2.5

CTI-BRM STIMULATED MONOCYTE CONDITIONED MEDIUM (IMCM) WAS PREPARED AS DESCRIBED IN SPECIFICATION. IMCM WAS ADDED AT A CONCENTRATION PREVIOUSLY DETERMINED TO INDUCE APPROXIMATELY 50% COLONY DIFFERENTIATION. IMCM AND ARA-C WERE ADDED DIRECTLY TO THE PLATES PRIOR TO ADDING THE MIA C51 CELL SUSPENSION. RESULTS ARE EXPRESSED AS AVERAGE OF TRIPLICATES ± SEM.

FIG._2

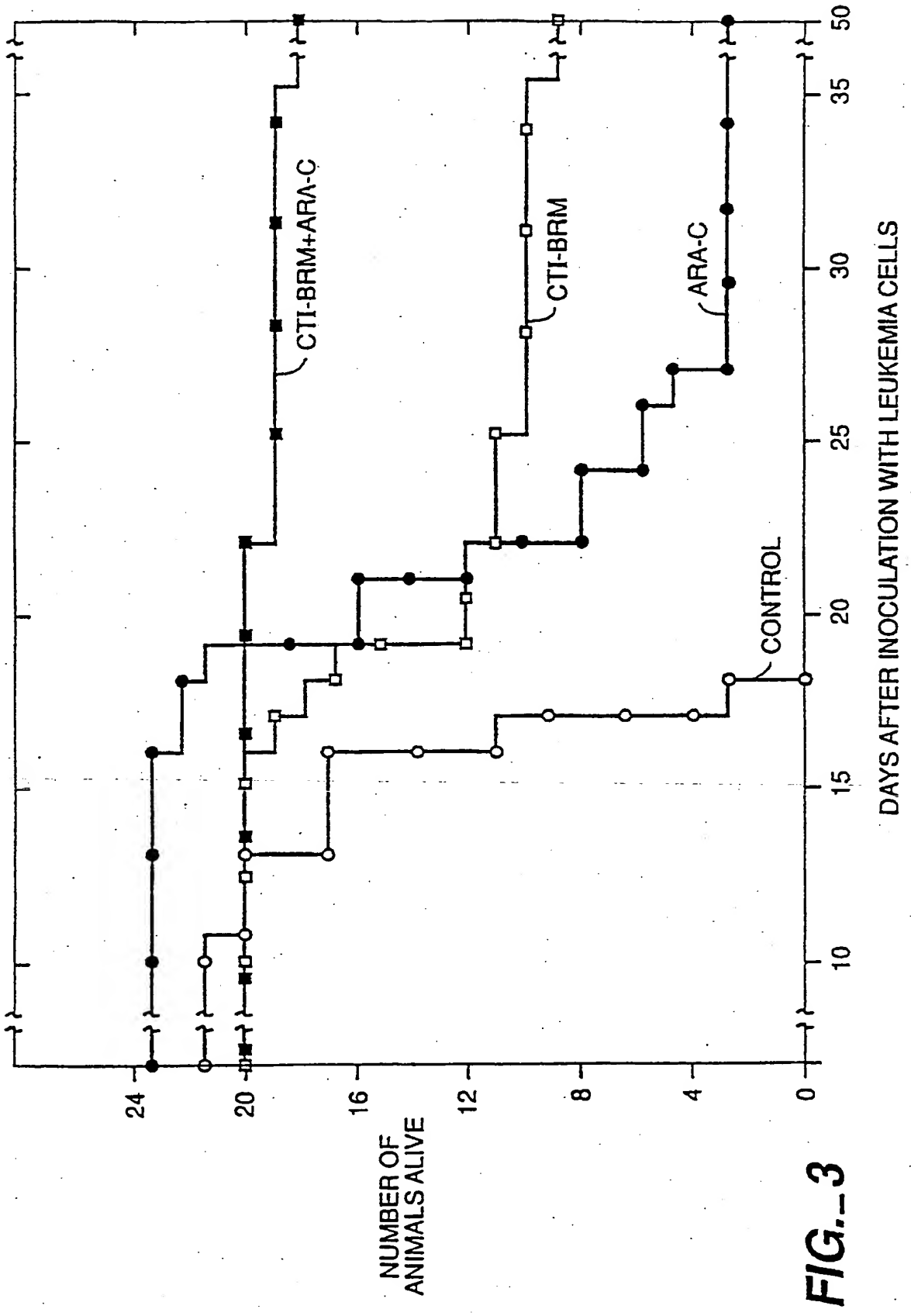

FIG._3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 91/01433

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/00
U.S.Cl. 424/88

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/450, 424/88; 514/49, 514/885; 435/881

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	WO, A, WO87/07503 (CELL TECHNOLOGY, INC.) 17 DECEMBER 1987 see the abstract, page 4, lines 22-29; pages 33-35, page 37, line 28 and page 29, line 4.	1-5, 7
Y	JAY S. ROTH, "ALL ABOUT CANCER", published in 1985, by GEORGE F. STICKLEY Company (PHILADELPHIA) see pages 166 and 167.	1-7
Y	CHARLES E. KUPCHELLA, "DIMENSIONS OF CANCER", published in 1987 by Wadsworth Publishing Company (BELMONT) see pages 311 and 315.	6
Y	US, A, 4,863,969 (BOLLAG) 05 SEPTEMBER 1989 see column 4, lines 61-68.	1-7

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
28 March 1991	15 MAY 1991
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Gollamudi S. Kishore

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☒ Claim numbers 8 . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

The claim fails to comply with the prescribed requirements in that it is a "use" claim which is indefinite. PCT Article 17(2)(b).

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:.

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.